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Preliminary phytochemical screening and antimicrobial activities of plant extract of *Elaeocarpus ganitrus* Roxb.

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Abstract: The present study was carried out for phytochemical screening of principle bioactive compounds and antimicrobial activity in *Elaeocarpus ganitrus* Roxb., Phytochemical analysis revealed the presence of saponin, terpenoid, steroid, saponin, flavonoid, tannin and alkaloid. The petroleum, ether, chloroform, methanol, acetone and aqueous extracts were subjected to antimicrobial activity against bacterial strains *Staphylococcus aureus, Pseudomonas, E. coli* and *Bacillus subtilis* against anti-fungal strains *A.awamori, A.fumigatus, Rhizopus oryzae, Trichoderma viridae* and *C.oryzae.* The antibacterial and antifungal activity was evaluated by disc-diffusion method.

Key words: *Elaeocarpus ganitrus* Roxb.; Anitfungal activity; Antibacterial activity; Disc-diffusion method

Introduction

Medicinal plants are of great value to mankind and society in general (Hill A.F, 1952). A broad range of medicinal plant parts, like root, stem, flower, fruit, twigs exudates and modified plant organs is used for extract as raw drugs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal S K., Singh K. N., Jamwal P, Lal B., 2006).

The use of medicinal plants as a source for relief from illness can be need back over five millennia to written documents of the early civilization in china, India and the near cast, but it is doubtless an art as old as mankind (Thomson War., 1978). With estimated. With estimated 2, 50, 000-5, 00, 000 plant species only a small percentage has been investigated photochemical which indicates the vast potential of higher plants as a source for new drugs (Mahesh B, Satish S, 2008).

Plants produce many compounds with complex molecular structures by a secondary metabolism some of these compounds and their derivatives are found to provide a rich source of botanicals, anthelmintics, and insecticides (Simoes C.M.O., *et al.,* 1999, Acharya S, Dash GK, Chhetrre R R. 2011). The chemical constituents of plants is very important, not only for the discovery of drugs and other therapeutic agents, but also in disclosing new sources of such economic materials as tannins, oils, gums precursors for the synthesis of complex chemical substances (Mojab F, Kamlimejad M, Ghaderi N, and Vahidipour H., 2003). Since many infectious microorganisms are resistant to synthetic drug, an alternative therapy is very much needed to attract the attention of many researchers all over the world (Mohanan PV, Rao JM, Kutti MAS, and Devi K S., 1998). Random screening as tool in discovering new biologically active molecules from medicinal plants has been most productive in the area of antibiotics and may give a new source of antimicrobial agents with possibly novel mechanisms or action (Mahesh B, Satish S, 2008, Barbour E K *et al.,* 2004, Motsei M L., *et al.,* 2003). Moreover, antimicrobials of plant origin are not associated with many side effects 13. Similarly, the importance of some plant extracts having anthelmintic activity cannot be ignored. The active components of herbal remedies can be combined with many inactive substances, increasing the safety and efficiency of the plant than that of its isolated and pure active components (Kamba A S, and Hassan L G., 2010). The photochemical constituents such, as alkaloids, flavonoids, isoflavonoids, saponins cumarins, glycosides, terpenes and phenolic compounds are transposable for antibacterial activity (Simoes C.M.O., *et al.,* 1999). Similarly, some investigators have mentioned the Importance of some phytochemicals like alkaloids, flavonoids, tannins, terpenoids and glycosides in imparting anthelmintic activity (Acharya S, *et al.,* 2008).

Elaeocarpus ganitrus commonly known as Rudraksha in India belongs to the Elaeocarpaceae family and grows in the Himalayan region (Dennis TJ, *Rudraksha* 1993). *Elaeocarpus* has about 360 species,

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occurs during Australia, East Asia, Malaysia and the Pacific Islands. About 120 species belonging to this genus from different parts of Asia and out of this, 25 species occur in India alone (Rauniar G P, and Sharma M, 2012). According to Hindu mythology, Rudraksha beads bear a great religion, spiritual, and materialistic significance. The Hindu mythology considers Rudraksha as symbol of link between earth and heaven. It is believed that it contains the secrets of evolution of entire cosmos within itself (Chaturvedi B K, Shiv Purana, 2004).

Elaeocarpus ganitrus Roxb. (Syn. *E. angustifolius Blume*, *E. sphaericus Gaertn.*) is an evergreen tree, with a spreading attractive crown, found in tropical and subtropical areas at the altitude ranging from the sea coast to 2, 000 meters more than the sea level. *E.ganitrus* found in Assam, Bihar, Bengal, Maharashtra, Madhya Pradesh and Sikkim in India (Sharma PV, 1995., Vaidyaratnam P S., 2005). Tree of Rudraksha is common along the foothills of all districts of Arunachal Pradesh, except tawang and upper subansiri and some other high-altitude areas. Tree of Rudraksha is originated in humid evergreen forests, which are characterized by three-tier forest structure (Bhuyan P, Khan M L, Tripathi R S 2002). It is a large evergreen tree with large leaves. Its height ranges from 50–200 feet. Leaves are large and shining green on the sun facing side and dull stringy on earth facing side. Flowers become visible in the month of April-May and are white or yellow in colour (Sharma PV, 1995., Vaidyaratnam P. S., 2005). Fruits start appearing in June and ripen near October. Ripe fruit is fleshy and has a seed with blue shell. Inner part or bead lying in the seed is called Rudraksha (Yelne, M. B., 1995).

Elaeocarpus ganitrus has an important position in Hindu religion and in Ayurveda, the ancient Indian system of medicine. Fruits of the plant are wearing by Hindu mystics as necklaces and in their daily worship for purpose of counting prayers and various magical properties are recognized to them (Singh B, Pal MSI, Sharma A, 2013). Rudraksha is the King of herbal medicine working effectively and positively, are measured to be sacred and have many spiritual and medicinal values both as defensive and remedial. It is used in folk medicine in treatment of stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases. According to the Ayurvedic medicinal system, wearing of Rudraksha can have a positive effect on nerves and heart (Gupta A, Aggarwal SS, and Basu D K, 1984, Sakat SS, *et al.,* 2009) As stated by Ayurvedic system of medicine, wearing Rudraksha beads relieves strain, anxiety, lack of concentration, insomnia, depression, hypertension, palpitation, infertility, rheumatism, and asthma (Nain J, Garg K, and Dhahiya S., 2012).

Materials and Methods

Plant Material

The medicinal plants *Elaeocarpus ganitrus* was selected and their parts like leaves, stems and fruits were collected around Haldwani, District Nainital, Uttarakhand, India, which is situated on piedmont grade called Bhabhar where the mountain rivers go underground to re-emerge in the Indo genetic plane, it has an average elevation of 424 meters and is located at 29.22 N and 79.52 E. The collected plant materials were brought to the laboratory for the study of antimicrobial activities and phytochemical analysis.

Extraction of plant material

Fresh field grown plants were collected and washed with running tap water followed by distilled water and thoroughly dipped in 70% ethanol for removing the adhered dust particles and disinfect. After blotting, the sample was air dried in shade, ground to fine powder and stored in clean air tight containers. The powdered mixture was then soaked in different solvents ethanol, methanol, acetone, petroleum ether and chloroform for 72 hrs. After filtering the contents using Whattman No 1 filter paper, the filtrate was left at room temperature for 48 hrs to evaporate partially. Greenish brown, dark green Light green, Yellowish green and greenish black residues were obtained. All the extracts were dried in vacuum rotary evaporator at 40ºC under reduced pressure, weighed and stored at 4ºC.

Phytochemical studies

The antimicrobial fraction obtained was subjected to various qualitative tests for the identification of constituents like flavonoids, alkaloids, saponins, steroid, terpenes, glycosides coumarin, reducing sugar, phenol and carotenoids.

Test for flavonoids

A few chop of 1 % NH3 solution was added to 1 ml of the antimicrobial fraction in a test tube. The appearance of yellow coloration shows the presence of flavonoids compound (Andzouana and Mombouli, 2011).

Test for reducing sugars

Take a test tube and add 2 ml of crude plant extract and add 5 ml of Distill water and filter. The filtrate was boiled with 3-4 drops of fehlings solution A and B for 2 minutes. Observe for orange red precipitate which indicates the presence of reducing sugars.

Test for Steroids

To the plant extract add 2 ml of acetic anhydride and add 0.5 gm of ethanolic extract of each sample with 2 ml of Sulphuric acid. Observe for the color change from violet to blue or green in samples indicating the presence of steroids.

Test for alkaloids
A quantity of 2 ml of Drangendroff's reagent was added to 1 ml of the antimicrobial fraction. The appearance of a turbid orange color shows the presence of alkaloids (Veerachari and Bopaiah, 2011).

Test for carotenoids
A quantity of 1 ml of the antimicrobial fraction was put in a test tube and dried under Fume Hood. A quantity of 10 ml of chloroform was added to the residue obtained and shaken vigorously. The resulting mixture was filtered and 85% sulphuric acid was added. The appearance of a blue color at the interface shows the presence of carotenoids (Ajayi *et al.,* 2011).

Test for saponins
A quantity of 10 ml of the antimicrobial fraction was shaken vigorously, sat aside for 10 min. The appearance of a stable froth shows the presence of saponins (Veerachari and Bopaiah, 2011).

Test for Phenol Compound
Ferric chloride test: the extract (50mg) was dissolved in 5 mL of distil led water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenol compounds.

Lead acetate test: the extract (50mg) was dissolved in 5 mL of distil led water. To this, 3ml of 10% lead acetate were added. A bulky white precipitate indicated the presence of phenol compound.

Test for glycosides
To 1 ml of the antimicrobial fraction, 1 ml of $FeCl_3$ reagent (mixture of 1 volume of 5% $FeCl_3$ solution + 99 volume of glacial acetic acid) and a few drops of concentrated H_2SO_4 were added. The appearance of a greenish blue color within few minutes shows the presence of glycosides (Trease and Evans, 1989).

Antimicrobial activity
Test microorganisms
Antibacterial assay was carried out on *Bacillus subtilis (gram +ve), Staphylococcus aureus (gram +ve), Pseudomonas (gram –ve), E.coli (gram -ve)* were procured from American Type Culture Collection (ATCC), and Microbial Type Culture Collection (MTCC) Institutes. All the organisms were sub cultured and maintained on nutrient media at 37℃.

Antifungal assay was conducted on *Aspergillus fumigatus, Rhizopus oryzae, Culbularia oryzae, Tricoderma virid, Aspergillus awamori.* were procured from American Type culture collection (ATCC), and Microbial Type Culture Collection (MTCC) Institutes. Fungal cultures were maintained on Sabouraud dextrose agar at 30℃.

Determination of Antibacterial and Antifungal Activity
The antibacterial and antifungal activity of all the solvent extracts of *Elaeocarpus ganitrus* Roxb. (stems and leaves) was evaluated by disc-diffusion method. When media is solidified or set, the disc (6 mm) of whattman no 1 filter paper was soaked in crude solvent viz. methanol, ethanol, chloroform, petroleum ether, and acetone and placed carefully in the center of Petri plates containing the solidified media. To compare the antimicrobial activity same concentration of the solvent using disc is placed in plate which acts as control to our crude solvents. Same procedure applied for the remaining Petri plates (for different solvents). The plates were incubated at 37 ^{0}C for 24 hrs for bacterial culture and for fungal culture the plates were incubated at 28^{0}C for 48 hrs. The plates were observed for inhibition of bacterial growth that was indicated by the clear zone around the well. The size of zone of inhibition (including well) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity. All experiments were carried out in triplicates.

Results and Discussion

Phytochemical analysis
The preliminary phytochemical test revealed that leaf crude extracts of *Elaeocarpus ganitrus* contains all the phytochemical constituents like reducing sugar, saponins, steroids, coumarin, and phenol. The stem extract contains steroids and coumarin, while the preliminary phytochemical test from two different solvents of stem crude extracts showed that *E.ganitrus* stem contain phytochemical constituents like reducing sugar, saponins, coumarin, phenol and except steroid.

Phytochecmical constituent	Leaves extract	Stem extract
Steroid	+	_
Reducing sugar	+	+
Phenol	+	+
Coumarin	_	+
Saponins	+	+

Antibacterial and Antifungal Activity
The results of the antimicrobial activity tests of crude extracts are shown in table 1. It was found that ten crude extracts of *Elaeocarpus ganitrus* at 100mg/mL concentration exhibited various antibacterial and antifungal activity.

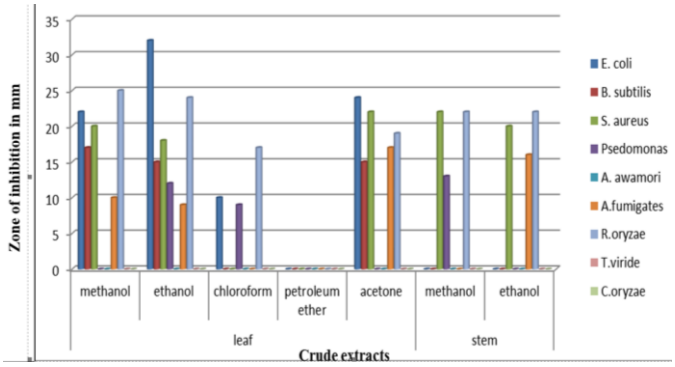
According to table the leaf crude extract was more effective against the bacterial strain in comparison to stem crude extract. Further analysis showed that from various crude extracts of leaf, methanolic and ethanolic crude extracts were more effective followed by acetone and chloroform while least activity was recorded by petroleum ether. In case

of stem methanol crude extract was more sufficient to inhibit the bacterial growth followed by methanol.

Table 1:

Crude Solvents	Leaf Extracts					Stem Extracts	
Pathogenic Microorganisms	MT	ET	PE	CF	AC	MT	ET
Gram positive Bacteria							
B.subtilis	17	15	0	0	15	0	10
S.aureus	20	18	0	0	22	22	20
Gram negative Bacteria							
Pseudomonas spp.	0	12	0	9	0	13	0
E. coli	22	32	0	10	24	0	0
Fungi							
A.fumigates	10	9	0	0	17	0	16
Rhizopus oryzae	25	24	0	17	19	22	22
Curvularia oryzae	0	0	0	0	0	0	0
Trichoderma viride	0	0	0	0	0	0	0
A.awamori	0	0	0	0	0	0	0

CF- Chloroform, MT- Methanol, ET- Ethanol, PE- Petroleum ether, AC-Acetone; Zone of inhibition in mm



Screening of antibacterial activity of leaf showed that it is potent antibacterial agent against *S.aureus, E. coli, Pseudomonas spp* and *B. subtilis*. Ethanol extract showed maximum activity, while crude extract in petroleum ether showed minimum activity against all the bacterial strains. Crude extracts of stem showed that it is potent antibacterial agent against *S.aureus* and *S.typhii*. Maximum activity was shown by ethanol and petroleum ether crude extract against *S.aureus*, while none of the crude extract was able to inhibit the growth of *B.subtilis and Pseudomonas*.

The leaf and stem maximum activity was found against *R. oryzae* followed by *A. fumigates*. Further investigation revealed that none of the crude extract of leaves and stem was able to inhibit the growth of *Curvularia oryzae, T. viride, A. awamori*. Bhaskara Rao *et al.,* (2011) also evaluated Antifungal activity of the extract against three molds (*Penicillium* sp, *A. niger* and *A. flavus*) and two yeast (*C. albicans* and *C. tropicalis*), isolated from clinical samples. Extract exhibited antifungal activity against all the fungal cultures except *A. niger*.

Conclusion

The plant *Elaeocarpus ganitrus* exhibited the presence of many secondary metabolites and revealed broad antimicrobial activity on the tested microorganisms. This investigation strongly suggests the possibility of this plant as an important source of antimicrobial drug development.

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